

Analysis of Eagle Egg Samples for PCDDs, PCDFs, and PCBs for Assessing Pollutant Bioaccumulation from the Columbia River Basin

**For U.S. Environmental Protection Agency
Region X**

**MRI Project No. 3189-A
Purchase Order No. 2y1154ntnx**

January 11, 1993

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**For U.S. Environmental Protection Agency
Region X
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Seattle, WA 98101**

**Attn: Mr. Robert Melton
Ms. Patricia Dooley**

**MRI Project No. 3189-A
Purchase Order No. 2y1154ntnx**

January 11, 1993

U.S. Environmental Protection Agency
Region X
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COVER PAGE - PCDD/PCDF/PCB ANALYSES DATA PACKAGE

Lab Name: Midwest Research Institute Contract No.: P02y 1158 n + n x SAS No.: N/A

Episode No.: N/A EPA Method No.: 1613, 8081 Method Issue/Rev. Date: 2/91
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Industrial Category: _____ Program: N/A

EPA Sample No.

Lab Sample ID

9111 0122

11998

9111 0123

11999

9111 0124

12000

Comments: Narrative Report is attached. X (Yes)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, for other than the conditions detailed in the Narrative Report. Release of the data contained in this hardcopy data package (and in the data submitted on magnetic media, if data is submitted on magnetic media), has been authorized by the Laboratory Manager or the Manager's designee, as verified by the following signature.

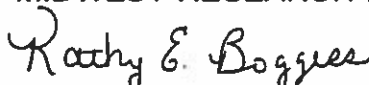
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PREFACE

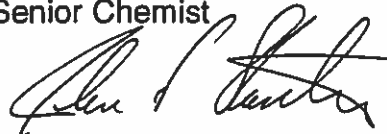
This final report provides the results of the analysis of eagle egg samples for polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). Congener-specific coplanar PCBs results and total PCBs based on Aroclor quantitation are presented. The results for 2,3,7,8-specific PCDDs and PCDFs and the results for total tetra through octa PCDD and PCDF homologs are included.

Midwest Research Institute (MRI) performed these analyses for the U.S. Environmental Protection Agency, Region X, Seattle, Washington. The samples were prepared for analysis by Ms. Sherry Wilner and Ms. Rose Schimmel. The HRGC/HRMS analyses and data reduction were performed by Mr. Robert Conklin and Mr. Mark Horrigan. Ms. Kathy Boggess supervised the sample preparation and analysis activities, reviewed the analytical data, and prepared this report.

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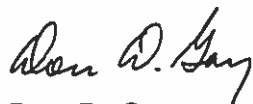


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SECTION 1

INTRODUCTION

Midwest Research Institute (MRI) was contracted by the U.S. Environmental Protection Agency, Seattle, WA, to determine the levels of PCDDs, PCDFs, and PCBs in three eagle egg samples. The samples were submitted to MRI for assessing pollutant bioaccumulation in an endangered species from the Columbia River Basin Study area.

The technical approach and scope of work were presented to Ms. Patricia Dooley, Regional Sample Control Center, in MRI Proposal 0912-090, dated August 6, 1992. A statement of work, dated August 12, 1992, was subsequently received from EPA Region X and comments were submitted by MRI to Ms. Dooley by telefax on August 20, 1992. A revised scope of work was received with authorization for MRI to perform the requested analysis as Purchase Order No. 2y1154ntnx, dated September 16, 1992.

Because the egg samples were obtained from an endangered species, MRI requested permission from the Fish and Wildlife service to possess and analyze the samples. In a letter to Kathy Boggess from Tami Tate-Hall, USFWS Permits, dated October 21, 1992, the U.S. Department of the Interior Fish and Wildlife Service granted authority for MRI to analyze the samples and to return the unused portions of the samples to EPA after completion of the study. Ms. Dooley arranged for shipment of the samples to MRI from USEPA, Duluth, Minnesota.

This report describes the methods used to prepare and analyze the eagle egg samples for PCDDs, PCDFs, and PCBs and presents the results for samples and quality control samples.

Section 2, entitled Experimental Approach discusses receipt of the samples by MRI, sample code assignments, analytical standards, PCDD/PCDF sample preparation procedures, PCB sample preparation procedures, HRGC/HRMS analysis, HRGC/ECD analysis, and data reduction.

Section 3 presents the sample results and the internal quality control results. Quality control data include the results of method blanks, the results of quality control eggs fortified with known levels of PCDDs, PCDFs, and PCBs, and the percent recoveries for the carbon-13 internal quantitation standards and surrogate standards. The organization of the data packet accompanying this report is discussed in Section 4.

SECTION 2

EXPERIMENTAL

This section describes sample receipt, analytical standards, sample preparation procedures, instrumental analysis, and data reduction.

2.1 SAMPLE RECEIPT

The sample collection study design and shipment of samples to MRI were coordinated by Ms. Patricia Dooley, USEPA, Seattle, Washington, and Mr. Brian Butterworth, USEPA, Duluth, Minnesota. The samples were received at MRI frozen and in good condition on October 20, 1992. However, five samples rather than three were received. Samples were received in glass jars as a mixture of egg material with no shell. It was not possible to separate the yolk from the white as presented in the scope of work.

Ms. Dooley was notified for clarification of sample identification for analysis, and on November 5, 1992, MRI was given verbal instructions to analyze sample codes 91110122, 91110123, and 91110124. MRI was instructed to hold the other two samples (91110125 and 91110126) for return to Fish and Wildlife after completion of the study. MRI will return these specimens upon notification by EPA Region X.

2.2 ANALYTICAL STANDARDS

The analytical standards included native PCDD, PCDF, and PCB isomers and their corresponding stable isotopes (carbon-13) for HRGC/HRMS analysis. The HRGC/ECD analysis required Aroclor standards, and surrogate standards, tetrachloro-*m*-xylene and decachlorobiphenyl. The PCDD, PCDF, and PCB standards were purchased from Cambridge Isotope Laboratories, Woburn Massachusetts, and Ultra Scientific, Hope, Rhode Island. The Aroclor standards were obtained as EPA reference standards, and the surrogates were purchased from Accustandard, New Haven, Connecticut.

2.2.1 PCDD/PCDF Standards

The native PCDD/PCDF and $^{13}\text{C}_{12}$ PCDD/PCDF isotopes were verified and documented in "Analysis for PCDDs and PCDFs in Human Adipose Tissue: Method Evaluation Study," J. S. Stanley et al., 1986.

The native 2,3,7,8-TCDD was received from the EPA Reference Materials Branch as a solution in isooctane. The other native PCDD and PCDF isomers were received from Cambridge Isotope Laboratories (CIL) as 1-mg neat standards (98% to 99% purity). Concentrated stock solutions of the individual PCDD and PCDF congeners were prepared from the neat standards, and dilutions were made with toluene. A concentrated working solution containing 17 native PCDD/PCDF isomers was prepared in toluene.

The $^{13}\text{C}_{12}$ internal quantitation stock standards (98% to 99% purity) were obtained from CIL. A concentrated working solution containing the nine internal quantitation standards was prepared. A separate working solution containing the two $^{13}\text{C}_{12}$ recovery standards was also prepared.

Aliquots of the native standard working solution, $^{13}\text{C}_{12}$ internal quantitation standard solution, and $^{13}\text{C}_{12}$ recovery standards solution were combined to prepare instrument calibration standards.

A secondary dilution of the native working solution was prepared for spiking the quality control method spike samples. Secondary dilutions of the $^{13}\text{C}_{12}$ internal quantitation standards and internal recovery standards were prepared for sample spiking solutions used for the quantitation of the PCDD and PCDF isomers.

The concentrations of the calibration standards were verified relative to a reference standard which was previously evaluated by MRI through an interlaboratory study. The reference standard was prepared from stock solutions of native PCDD and PCDF isomers supplied by CIL for use in an interlaboratory evaluation of the CIL standards. The individual stock solutions (50 ng/ μL) were combined to prepare a working solution, and an aliquot of the working solution was combined with the $^{13}\text{C}_{12}$ internal quantitation standards working solution to prepare a calibration check standard. The results of the interlaboratory evaluation of the CIL reference standard were presented at the 1987 dioxin conference, J. C. Bradley et al., 1987.

2.2.2 PCB Standards

The coplanar PCB (congeners 77, 126, 169) analytical standards were prepared from the neat native standards (99% purity EPA reference standards) diluted in isooctane. Mixtures of the native stock solutions were combined to prepare a native working solution.

The corresponding carbon-13 labeled coplanar PCB congeners 126 and 169 were obtained as solutions in nonane, and the carbon-13 PCB 77 was synthesized at MRI (Roth et al., 1982). Mixtures of the isotopically labeled PCBs were combined to prepare an internal quantitation standard spiking solution. The native working solutions and $^{13}\text{C}_{12}$ working solutions were combined to prepare the HRGC/HRMS calibration standards.

The native coplanar PCB working solution was diluted to prepare a spiking solution for quality control method spikes. The concentration of the spiking solution was 10 pg/ μ L for each of the coplanar PCBs, and 40 μ L of this solution was used to fortify the matrix spike samples. A secondary dilution of the $^{13}\text{C}_{12}$ PCB working solution was performed to prepare the internal quantitation spiking solution (IQS) at a concentration of 2 pg/ μ L for each isomer, and each blood sample was spiked with 400 μ L of this solution.

The Aroclor 1260 stock standard was prepared by diluting neat Aroclor 1260 (100% purity, EPA) in toluene. Spiking solutions and instrument calibration standards were prepared by serial dilutions in isooctane. A stock spiking solution was prepared at a concentration of 34.6 $\mu\text{g/mL}$, and instrument calibration standards were prepared at concentrations of 0.2076, 0.346, 1.038, 2.076, and 3.46 $\mu\text{g/mL}$.

A surrogate spiking solution was prepared by dilution of the stock standard to a final concentration of 0.2 $\mu\text{g/mL}$ for tetrachloro-*m*-xylene and decachlorobiphenyls. Instrument calibrations were prepared from dilutions of the spiking solution to 0.0025, 0.0050, 0.0075, and 0.01 $\mu\text{g/mL}$.

The pentachlorobiphenyl congener 105 (2,3,3',4,4') was prepared for previous congener-specific PCB analyses conducted at MRI and was included as a mixture with other PCB congeners. The stock solution (1000 pg/ μ L) was diluted to 50 pg/ μ L and 250 pg/ μ L for instrument calibration standards.

2.3 SAMPLE PREPARATION

The analytical procedures used for the eagle egg analysis were evaluated at MRI through previous studies, including a PCDD/PCDF bioaccumulation study in chicken egg samples. The procedures used for sample preparation included the extraction techniques for fish tissue presented in EPA Method 8290 (November, 1990), and the cleanup procedures were modifications of procedures specified in EPA Method 8290. A schematic of the sample preparation procedure is shown in Figure 1.

Quality control samples prepared with the three eagle egg samples included a method blank, an unspiked quality control (QC) egg pool sample, a PCDD/PDDF and coplanar PCB spiked QC egg pool sample, and an Aroclor 1260 spiked QC egg pool sample. The QC egg pool consisting of composite chicken egg yolks was prepared and characterized during previous MRI studies.

Aliquots of the eagle egg samples and QC egg samples (10 g weighed to the nearest 0.01 g) were weighed into 250 mL beakers and mixed with sodium sulfate. A sodium sulfate method blank and the egg/sodium sulfate sample mixtures were fortified with $^{13}\text{C}_{12}$ -PCDD, $^{13}\text{C}_{12}$ -PCDF, and $^{13}\text{C}_{12}$ -PCB internal quantitation standards for HRGC/HRMS analysis and tetrachloro-*m*-xylene and decachlorobiphenyl surrogates for HRGC/ECD analysis. The spiking solutions are listed in Table 1.

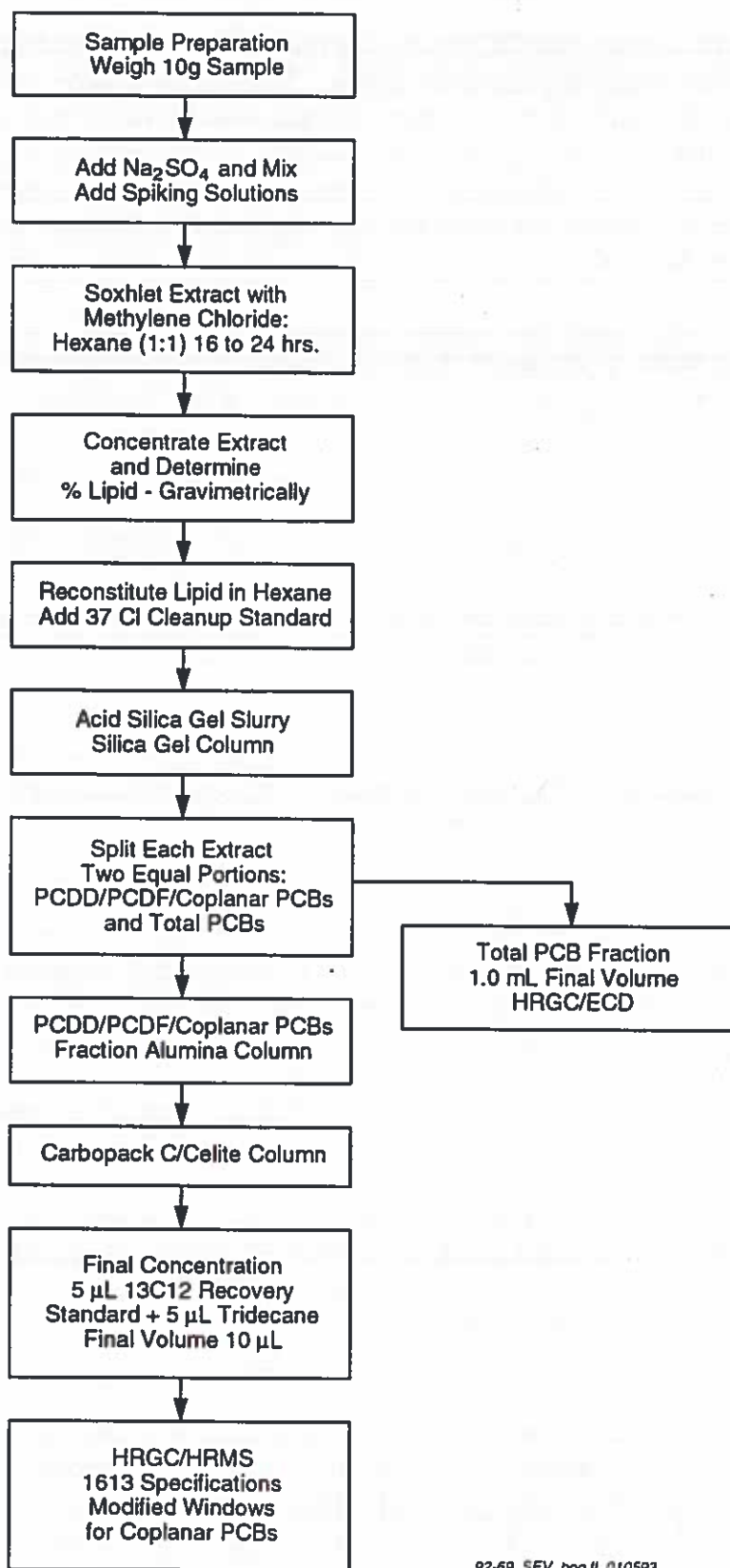


Figure 1. Sample preparation and analysis scheme.

TABLE 1. PCDD/PCDF AND PCB SPIKING SOLUTIONS

Compound	Concentration pg/ μ L	Volume spiked (μ L)	Amount spiked (pg)
¹³C-PCDD/PCDFIQS			
¹³ C-2,3,7,8-TCDF	40	50	2000
¹³ C-2,3,7,8-TCDD	40		2000
¹³ C-1,2,3,7,8-PeCDF	40		2000
¹³ C-2,3,4,7,8-PeCDF	40		2000
¹³ C-1,2,3,7,8-PeCDD	40		2000
¹³ C-1,2,3,4,7,8-HxCDF	40		2000
¹³ C-1,2,3,6,7,8-HxCDF	40		2000
¹³ C-2,3,4,6,7,8-HxCDF	40		2000
¹³ C-1,2,3,7,8,9-HxCDF	40		2000
¹³ C-1,2,3,4,7,8-HxCDD	40		2000
¹³ C-1,2,3,6,7,8-HxCDD	40		2000
¹³ C-1,2,3,4,6,7,8-HpCDF	40		2000
¹³ C-1,2,3,4,7,8,9-HpCDF	40		2000
¹³ C-1,2,3,4,6,7,8-HpCDD	40		2000
¹³ C ₁₂ -OCDD	80		4000
¹³C Coplanar PCBs			
¹³ C ₁₂ -PCB 77	2	400	800
¹³ C ₁₂ -PCB 126	2		800
¹³ C ₁₂ -PCB 169	2		800
Surrogates			
Tetrachloroxylene	200	200	40000
Decachlorobiphenyl	200		
Cleanup Standard			
³⁷ Cl-2,3,7,8-TCDD	4	50	200
Internal Recovery Standard			
¹³ C-1,2,3,4-TCDD	200	5	1000
¹³ C-1,2,3,7,8,9-HxCDD	200		1000

In addition to the $^{13}\text{C}_{12}$ standards and surrogate standards, one aliquot of the QC egg pool material was fortified with native PCDD, PCDF, and coplanar PCB standards, and a separate aliquot of QC egg pool was fortified with Aroclor 1260. The concentrations of the native spiking solutions are shown in Table 2.

The eagle egg samples and quality control samples were placed in Soxhlet extractors and extracted for 16 h with a 50:50 mixture of methylene chloride:hexane. The extracts were cooled to ambient temperature, filtered through a bed of sodium sulfate, and transferred to preweighed 500-mL boiling flasks for concentration of the solvent by rotary evaporation. After the solvent was removed, the weight of the lipid residue remaining in the flask was determined and the percent lipid was calculated.

The lipid residue was diluted in hexane and 50 μL of a ^{37}Cl -2,3,7,8-TCDD cleanup standard (4 pg/ μL) was added to each extract. The samples were subjected to a sulfuric acid modified silica gel slurry and neutral/acid silica gel chromatography column cleanup described in EPA Method 8290. After this step, the volume of each extract was adjusted to 10.0 mL and split into two equal 5.0 mL portions. One portion was designated as the total PCB portion and concentrated to a final volume of 1.0 mL. The other portion, designated as the PCDD/PCDF/Coplanar PCB portion, was put through additional cleanup steps. Subsequent column chromatography cleanup steps included neutral alumina and Carbowack C/Celite which are described in EPA Method 8290.

Following the final cleanup, the PCDD/PCDF coplanar PCB extracts were concentrated under prepurified nitrogen to 100 μL and 5 μL of the recovery standard solution (Table 1) in tridecane was added in addition to 5 μL tridecane. The evaporation was continued until a volume of 10 μL was reached. Sample extracts were transferred to refrigerated storage (4°C) until HRGC/HRMS analysis was initiated.

2.4 HRGC/HRMS ANALYSIS- PCDDs, PCDFs, AND COPLANAR PCBs

The analysis of the eagle egg samples for coplanar PCBs (Congeners 77, 126, and 169) was performed using the same analytical conditions specified for PCDDs and PCDFs. The coplanar PCBs are typically detected at concentrations an order of magnitude below the more prevalent mono and di-substituted PCBs. Because of these differences in concentrations, it was necessary to develop an analysis technique for coplanar PCBs separate from the ortho-PCB analysis.

The PCDD and PCDF analyses were performed according to EPA Method 1613 with modifications to include quantitation masses for tetra, penta, and hexa PCBs. The PCB masses used for quantitation are specified in EPA Method 680 and were modified to minimize interference from PeCDD. The quantitation masses for PeCDD and $^{13}\text{C}_{12}$ -PeCDD were modified to minimize interference from HxPCB. The quantitation masses are included in the data packet that accompanies this report.

TABLE 2. SPIKING SOLUTIONS ADDED TO QUALITY CONTROL
EGG POOL SAMPLES

Compound	Concentration pg/ μ L	QC Spike 1 volume spiked (μ L)	QC Spike 1 amount spiked (pg)	QC Spike 2 volume spiked (μ L)	QC Spike 2 amount spiked (ng)
2,3,7,8-TCDF	4	50	200		
2,3,7,8-TCDD	4		200		
1,2,3,7,8-PECDF	20		1000		
2,3,4,7,8-PECDF	20		1000		
1,2,3,7,8-PECDD	20		1000		
1,2,3,4,7,8-HXCDF	20		1000		
1,2,3,6,7,8-HXCDF	20		1000		
2,3,4,6,7,8-HXCDF	20		1000		
1,2,3,7,8,9-HXCDF	20		1000		
1,2,3,4,7,8-HXCDD	20		1000		
1,2,3,6,7,8-HXCDD	20		1000		
1,2,3,7,8,9-HXCDD	20		1000		
1,2,3,4,6,7,8-HPCDF	20		1000		
1,2,3,4,7,8,9-HPCDF	20		1000		
1,2,3,4,6,7,8-HPCDD	20		1000		
1,2,3,4,6,7,8,9-OCDF	40		2000		
1,2,3,4,6,7,8,9-OCDD	40		2000		
3,3',4,4'-Tetra PCB (#77)	10	40	400		
3,3',4,4',5-Penta PCB (#126)	10		400		
3,3',4,4',5,5'-Hexa PCB (#169)	10		400		
	ng/ μ L				
Aroclor 1260	34.6			20	692

The PCDD, PCDF, and coplanar PCB analyses were performed using a VG70 250 S HRMS with mass resolution > 10,000. Analytical parameters for the HRGC/HRMS determinations are given in Table 3.

The initial PCDD/PCDF calibration curve consisted of a series of five standards ranging in concentration from 0.5 to 200 pg/μL for the tetra isomers, 2.5 to 1000 pg/μL for the penta through hepta isomers, and 5 to 2000 pg/μL for the octa homologs. Table 4 gives the concentration for each of the isomers in the calibration standards.

The coplanar PCB calibration curve included a series of six standards ranging in concentration from 4 to 500 pg/μL. The concentration of the coplanar PCBs are given in Table 5.

The day that the eagle egg and QC samples were analyzed started with the mass calibration of the mass spectrometer, followed by the analysis of a window-defining mix which contains the first and last eluting isomers of a homolog group. This mix is used to determine the ion switching points needed to switch from one homolog series to the next. The mixture is also used to calculate separation of the 2,3,7,8-TCDD isomer from other closely eluting TCDD isomers. The 2,3,7,8 isomer was resolved from other TCDD isomer with a valley < 25 %.

Following the analysis of the window-defining mix, a midpoint level PCB calibration standard was analyzed to verify response factors consistent with the initial calibration curve analyzed previously. The five point PCDD/PCDF calibration curve was then analyzed, followed by a tridecane blank, the method blank, QC egg samples, and eagle egg samples.

2.5 MONO-ORTHO AND DI-ORTHO PCBs HRGC/ECD ANALYSIS

The analyses of the sample extracts for mono-ortho and di-ortho substituted congener specific PCBs were performed by high resolution gas chromatography (HRGC) using an Hewlett Packard 5890 II gas chromatograph equipped with an electron capture detector (ECD). The instrumental parameters for Aroclor quantitation are specified in Table 6. For individual congener quantitation (2,3,3',4,4'-Penta PCB Congener 105) the parameters were the same, except the oven temperature program was optimized for separation of close eluting PCB congeners.

The HRGC/ECD analyses for Aroclor analysis and surrogate recoveries were performed as an uninterrupted sequence using an autosampler injection system. The analysis order included an initial calibration curve for surrogates and Aroclors, followed by solvent blanks, method blanks, samples, and continuing calibration check standards. The samples were screened for Aroclor 1242, 1248, 1254, 1260, and 1268 based on MRI's previous experience with Aroclor patterns analyzed under the same conditions as the samples.

**TABLE 3. HRGC/HRMS OPERATING CONDITIONS FOR PCDD/PCDF
AND COPLANAR PCB ANALYSIS**

<u>Mass Spectrometer</u>	<u>VG70 250S</u>
Accelerating voltage:	8,000 V
Trap current:	500 μ A
Electron energy:	35 eV
Photo multiplier voltage:	320 V
Source temperature:	280°C
Resolution:	$\geq 10,000$ (10% valley definition)
Overall SIM cycle time:	1 s
<u>Gas Chromatograph</u>	
Column coating:	DB 5
Film thickness:	0.25 μ m
Column dimensions:	60 m x 0.25 mm i.d.
He linear velocity:	~ 25 cm/s
He head pressure:	1.75 kg/cm ² (25 psi)
Injection type:	Splitless, 45 s
Split flow:	30 mL/min
Purge flow:	6 mL/min
Injector temperature:	290°C
Interface temperature:	290°C
Injection size:	2 μ L
Initial temperature:	200°C
Initial time:	2 min
Temperature program:	200° to 270°C at 5°C/min
Second hold time:	10 min
Second temperature ramp:	270° to 330°C at 5°C/min
Final hold time:	5 min

TABLE 4. PCDD/PCDF HRGC/HRMS CALIBRATION CURVE STANDARDS (pg/μL)

Compound	CS1	CS2	CS3	CS4	CS5
Native PCDDs/PCDFs					
2,3,7,8-TCDF	0.5	2.0	10	40	200
2,3,7,8-TCDD	0.5	2.0	10	40	200
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8,9-OCDF	5.0	20	100	400	2000
1,2,3,4,6,7,8,9-OCDD	5.0	20	100	400	2000
Labeled IQS					
¹³ C-2,3,7,8-TCDF	100	100	100	100	100
¹³ C-2,3,7,8-TCDD	100	100	100	100	100
¹³ C-1,2,3,7,8-PeCDF	100	100	100	100	100
¹³ C-2,3,4,7,8-PeCDF	100	100	100	100	100
¹³ C-1,2,3,7,8-PeCDD	100	100	100	100	100
¹³ C-1,2,3,4,7,8-HxCDF	100	100	100	100	100
¹³ C-1,2,3,6,7,8-HxCDF	100	100	100	100	100
¹³ C-2,3,4,6,7,8-HxCDF	100	100	100	100	100
¹³ C-1,2,3,7,8,9-HxCDF	100	100	100	100	100
¹³ C-1,2,3,4,7,8-HxCDD	100	100	100	100	100
¹³ C-1,2,3,6,7,8-HxCDD	100	100	100	100	100
¹³ C-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
¹³ C-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
¹³ C-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
¹³ C ₁₂ -OCDD	100	100	100	100	100
Cleanup Standard					
³⁷ Cl-2,3,7,8-TCDD	0.5	2	10	40	200
Internal Recovery Standards					
¹³ C-1,2,3,4-TCDD	100	100	100	100	100
¹³ C-1,2,3,7,8,9-HxCDD	100	100	100	100	100

TABLE 5. COPLANAR PCB HRGC/HRMS CALIBRATION STANDARDS (pg/ μ L)

Compound	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6
3,3',4,4'-Tetra PCB (#77)	4	10	40	100	200	500
3,3',4,4',5-Penta PCB (#126)	4	10	40	100	200	500
3,3',4,4',5,5'-Hexa PCB (#169)	4	10	40	100	200	500
¹³ C-3,3',4,4'-Tetra PCB	40	40	40	40	40	40
¹³ C-3,3',4,4',5-Penta PCB	40	40	40	40	40	40
¹³ C-3,3',4,4',5,5'-Hexa PCB	40	40	40	40	40	40
¹³ C-1,2,3,4-TCDD	100	100	100	100	100	100

TABLE 6. GAS CHROMATOGRAPHIC CONDITIONS FOR HRGC/ECD ANALYSIS

Gas chromatograph: Hewlett-Packard 5890 Series II

Detector: ⁶³Ni electron capture detector

Column: 30 m, 0.32 mm i.d. DB-5

Aroclor

Column temperature: 90°C/1 min → 210°C/8 min at 30°C/min
Then → 280°C/15 min at 35°C/min

Individual Congener

Column temperature: 90°C/1 min → 210°C/1 min at 30°C/min
Then → 270°C/10 min at 1°C/min

Injector temperature: 270°C

Detector temperature: 320°C

Carrier gas: 2.5 mL/min helium

Makeup gas: 30 mL/min P-5 argon/methane

Injection volume: 2.0 μ L

Data system: 1. Nelson Analytical Model 4400 chromatography data system
2. HP 3396A integrator

Aroclor 1260 calibration standards were analyzed at concentrations of 0.2076, 0.346, 1.038, 2.076, and 3.460 µg/mL to bracket concentrations expected in the samples. An initial surrogate calibration curve was analyzed at concentrations of 0.0025, 0.0050, 0.0075, and 0.010 µg/mL for tetrachloro-*m*-xylene and decachlorobiphenyl corresponding to theoretical recoveries of 25%, 50%, 75%, and 100%.

The criterion for an acceptable calibration was precision of the calibration factors < 20% relative standard deviation. The criterion for continuing calibration was a calibration factor within 25% of the mean calibration factor from the initial calibration curve.

The HRGC/ECD analysis for PCB congener 105 was performed as a separate analysis from the Aroclor analysis. The oven temperature program was slowed for separation of congener 105 from the more prevalent congener 153. Calibration factors for congener 105 were determined over three concentration levels (50 ng/mL, 250 ng/mL, 1000 ng/mL), followed by analysis of the eagle egg samples and a calibration check standard.

2.6 PCDDs/PCDFs AND COPLANAR PCBs DATA REDUCTION

2.6.1 Qualitative

The data were reduced using a high-speed computer program that filters noise and calculates the responses of analytes in the appropriate mass windows with ion abundance ratios at $\pm 20\%$ of the theoretical ratios. Detected peaks must also fall within established relative retention time windows. Relative retention times of native isomers to the corresponding $^{13}\text{C}_{12}$ isomer for each homolog were established from the analysis of a calibration standard.

The PCDD and PCDF quantitation ions and theoretical ion abundance ratio criteria are specified in EPA Method 1613. The tetra-hexa coplanar PCB quantitation ions and theoretical ion abundance ratio criteria are specified in EPA Method 680.

2.6.2 Quantitation

For peaks that pass that qualitative criteria, the computer program calculates an extract concentration, and then the sample weights and extract volumes are taken into account to arrive at a final sample concentration.

Limits of detection for analytes not positively identified were based on the lowest calibration standard or by reporting the concentration of a coeluting peak that did not meet the qualitative ion ratio criteria.

2.6.3 PCDDs, PCDFs, and Coplanar PCBS—Calculation Formulas

The calculations for determining the relative response factors and the concentrations in the sample extracts are described in detail in EPA Methods 8290 and 1613.

The instrument was calibrated with the series of calibration standards presented in Table 5, and RRFs were determined for each native compound relative to the corresponding ¹³C-labeled internal quantitation standard (IQS) (Equation 1) and for each IQS relative to the recovery standard (RS) (Equation 2). The mean RRFs from all standards were then used in subsequent calculations to determine sample amounts for each specific isomer.

As discussed in the Sample Preparation Section, known amounts of IQS were added to the samples before extraction, and the IQS concentration in the final extract was used to calculate the concentration of the native analytes in the final extract as an isotope dilution calculation technique. This calculation procedure (Equation 3) adjusts for recovery from the sample matrix.

$$RRF = \frac{A_{STD} \times C_{IS}}{A_{IS} \times C_{STD}} \quad \text{Eq. 1}$$

where: A_{STD} = the sum of the area responses for the two characteristic ions of the native standard;
 A_{IS} = the sum of the area responses for the two characteristic ions of the corresponding internal quantitation standard;
 C_{IS} = concentration (pg/μL) of the internal quantitation standard; and
 C_{STD} = concentration (pg/μL) of the native standard.

$$RRF_{IS} = \frac{A_{IS} \times C_{RS}}{A_{RS} \times C_{IS}} \quad \text{Eq. 2}$$

where A_{IS} and C_{IS} are defined as in Equation 1 and

RRF_{IS} = the average of initial calibration response factors of the internal quantitation standard relative to the internal recovery standard,

C_{RS} = concentration (pg/μL) of the internal recovery standard, and

A_{RS} = the sum of the area responses for the two characteristic ions corresponding to the internal recovery standard.

$$C_{WT} = \frac{A_{\text{sample}} \times Q_{IS} \times V_e \times S}{A_{IS} \times RRF \times Wt} \quad \text{Eq. 3}$$

where: C_{WT} = lipid-based (pg/g) concentration of the PCDD or PCDF congener;

A_{sample} = sum of the area responses for the two characteristic ions of the PCDD or PCDF congener;

Q_{IS} = concentration (pg/ μ L) of the internal quantitation standard added to the sample;

V_e = final extract volume (μ L);

S = split factor = 2;

A_{IS} = sum of the area responses for the two characteristic ions of the respective internal quantitation standard;

RRF = the average of the initial calibration relative response factors for the PCDD or PCDF congener from Equation 1; and

Wt = amount of lipid (g) extracted from the sample.

$$\text{Recovery (\%)} = \frac{A_{IS} \times Q_{RS}}{A_{RS} \times RRF_{IS} \times Q_{IS}} \times 100 \quad \text{Eq. 4}$$

where: A_{RS} = sum of the area responses for the two characteristic ions of the internal recovery standard;

Q_{RS} = amount of the internal recovery standard added to the final extract; and

RRF_{IS} = the average of initial calibration response factors of the internal quantitation standard relative to the internal recovery standard.

The recovery standards which are added to the sample at the final concentration step are used to establish the absolute recovery of the carbon-13 internal standards (Equation 4). The IQS recoveries are used to assess overall method performance and adjust the results for native congeners.

2.6.4 PCDD/PCDF Total Homolog Data Reduction

Total homolog PCDD and PCDF results were determined in addition to 2,3,7,8 isomer-specific data. EPA Method 1613 was modified to determine levels of total tetra through hepta PCDDs, and PCDFs. For each homolog group, response factors were calculated based on the $^{13}\text{C}_{12}$ -internal quantitation standards specified in EPA Method 8290. For homolog groups having more than one 2,3,7,8 PCDD or PCDF standard, the average RF was calculated.

Quantitation windows for the non-2,3,7,8 PCDD and PCDF isomers were established from the analysis of the window-defining standard. For peaks that met the homolog-specific qualitative ion ratio criteria, responses were calculated relative to the homolog-specific response factor. For each homolog group, the peaks detected above the lowest calibration standard were summed.

2.7 HRGC/ECD AROCLOR DATA REDUCTION

The splits of the sample extracts designated for total PCB analysis were analyzed by HRGC/ECD, and patterns of peaks observed were compared to Aroclor specific chromatograms obtained under the same analytical conditions. Exact matches to Aroclor patterns are generally difficult to achieve in biological matrices.

The patterns of peaks observed in the three eagle egg samples were generally consistent with Aroclor 1260 standards. An interference peak at retention time 18.6 to 18.7 min shown in Figures 2 through 4 is attributed to chlorinated pesticides interferences, typically detected in biological samples. Based on these pattern matches, total PCB quantitation was performed relative to Aroclor 1260 quantitation standards.

The peak areas from 15.7 to 30.3 min in the Aroclor 1260 quantitation standards were summed and compared to the standard concentration. The large interference peak (18.6 to 18.7 min) was excluded from the window. A calibration factor (CF) was calculated as area/concentration. The mean calibration factor from the initial calibration curve was then used to quantitate Aroclor 1260 in the eagle egg samples.

The peak areas from 15.7 to 30.3 min were summed, excluding the interference peak at 18.6 to 18.7 min. The sample concentration was determined by dividing the summed area by the mean calibration factor and then multiplying by the dilution factor. The calculations are shown on spreadsheets in the accompanying data packet.

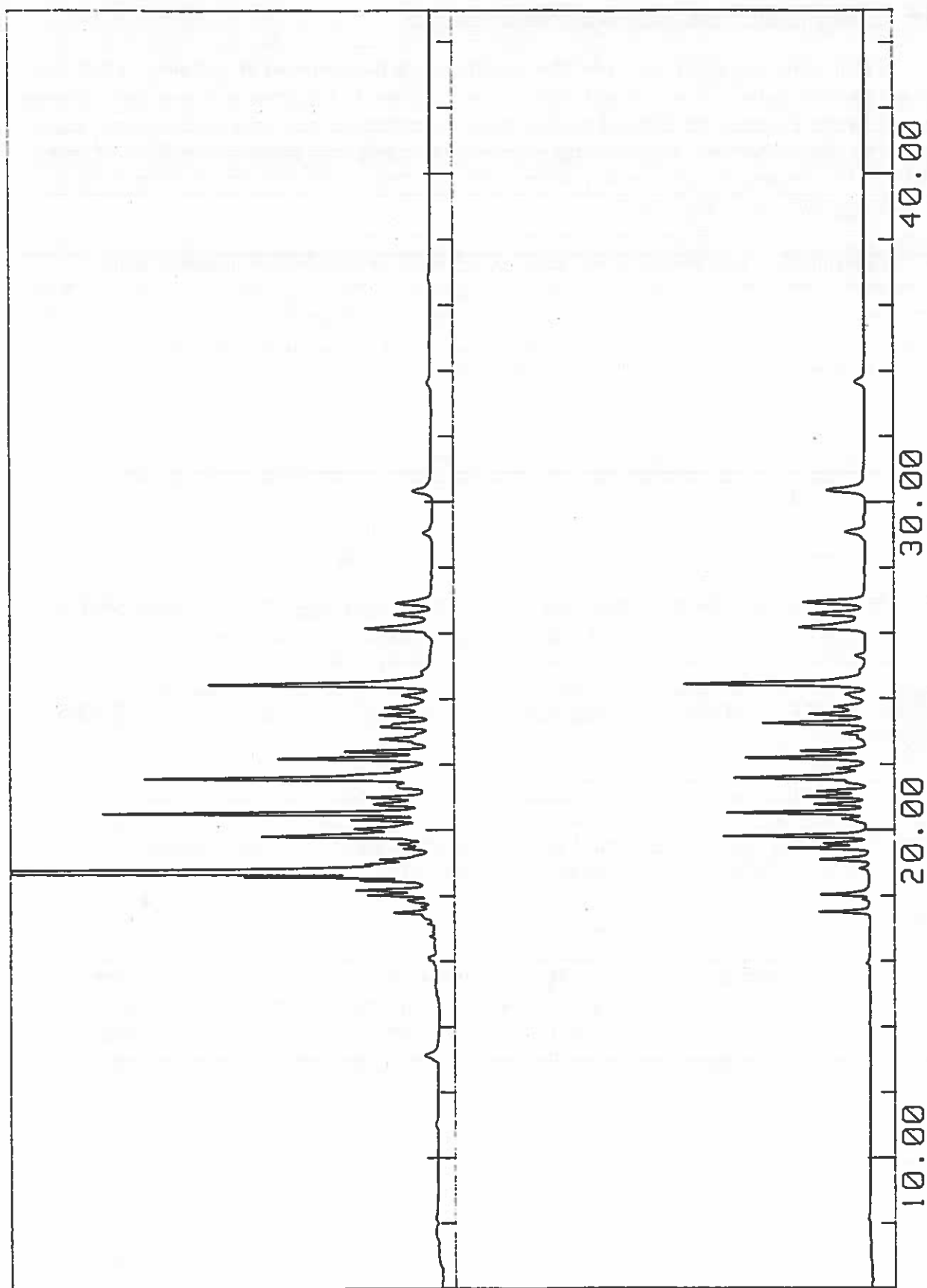


Figure 2. Eagle egg sample 91110122 (top) and Aroclor 1260 standard 2.076 µg/mL (bottom).

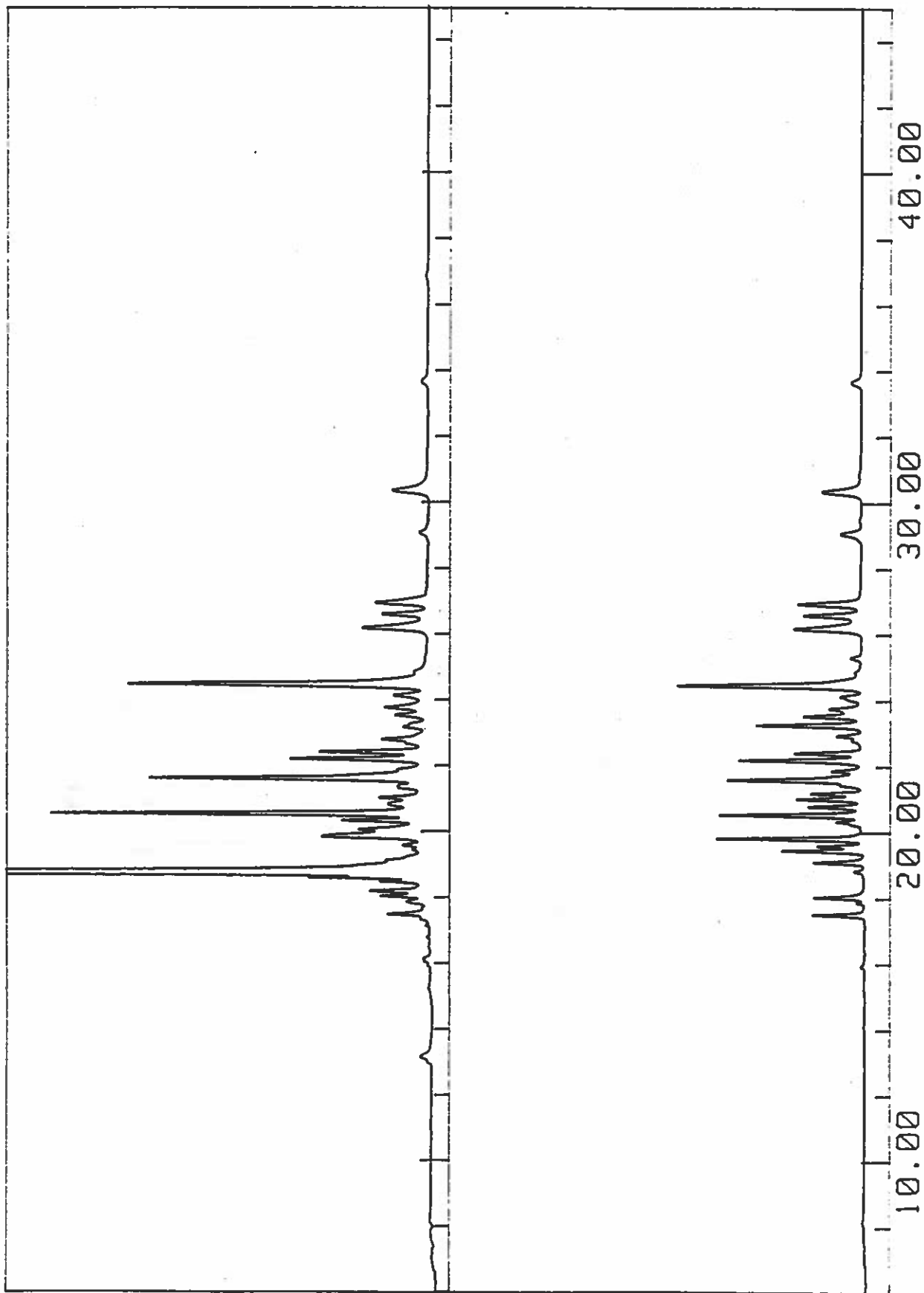


Figure 3. Eagle egg sample 91110123 (top) and Aroclor 1260 standard 2.076 µg/mL (bottom).

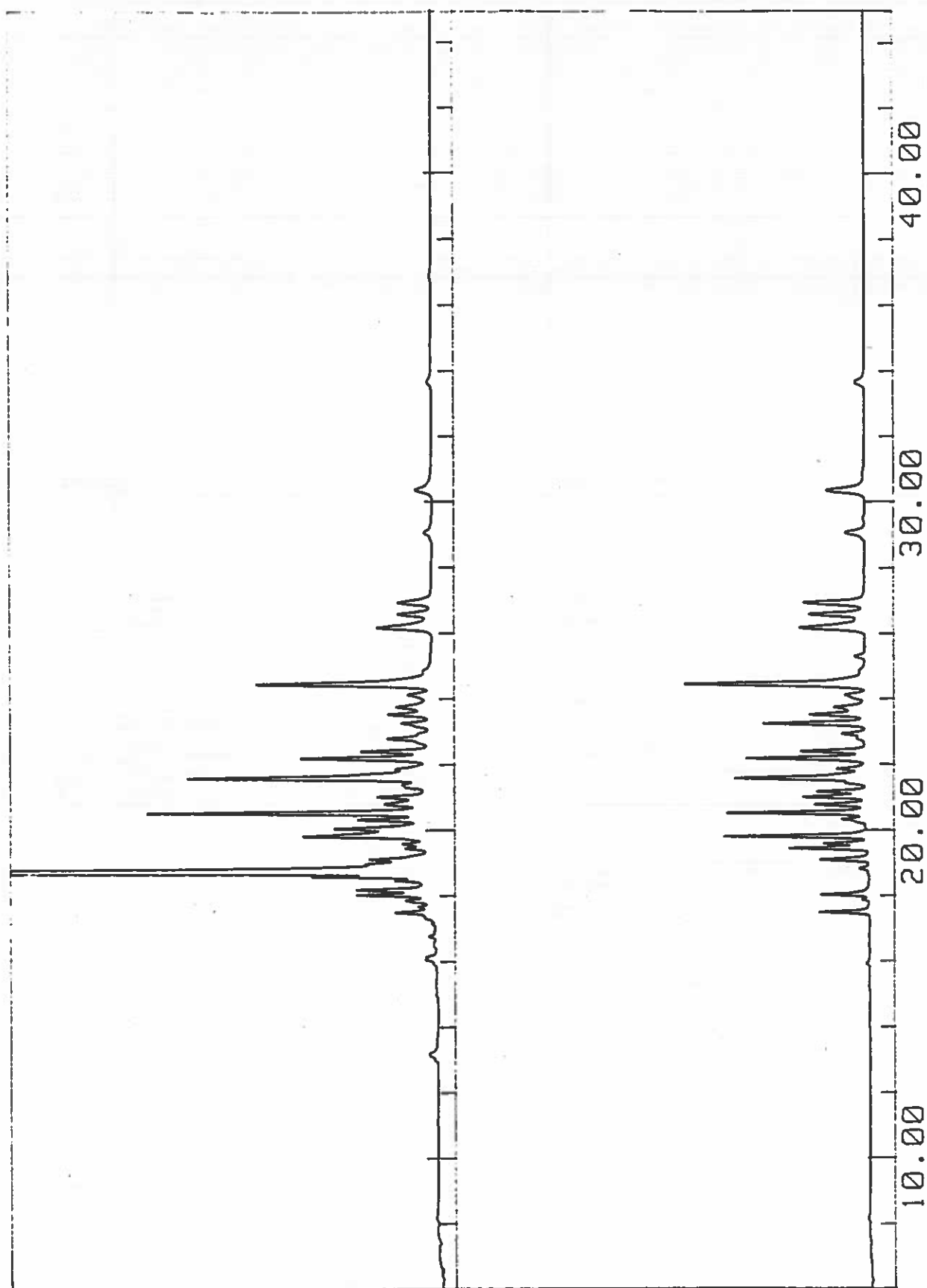


Figure 4. Eagle egg sample 91110124 (top) and Aroclor 1260 standard 2.076 $\mu\text{g/mL}$ (bottom).

2.8 HRGC/ECD CONGENER SPECIFIC PCB ANALYSIS

The analysis of PCB congener 105 was not included in the coplanar PCB analysis because this congener, a mono-ortho substituted PCB, would not recover through the carbon column cleanup used for PCDD, PCDF, and coplanar PCB analysis. Previous MRI studies have included congener specific analysis of PCB congener 105 with other more prevalent congeners. A slower oven program was used to optimize separation of the PCB congeners. As shown in Figure 5, congener 105 is observed after a major PCB congener 153 in sample 91110124. Figure 5 also shows the presence of other congener specific PCBs typically detected in biological matrices.

The area response for congener 105 was determined in an initial calibration standard, and calibration factors were determined. The area for congener 105 in the eagle samples was divided by the calibration factor, and dilution factors and sample weights were taken into account to yield concentration in units of ng/g.

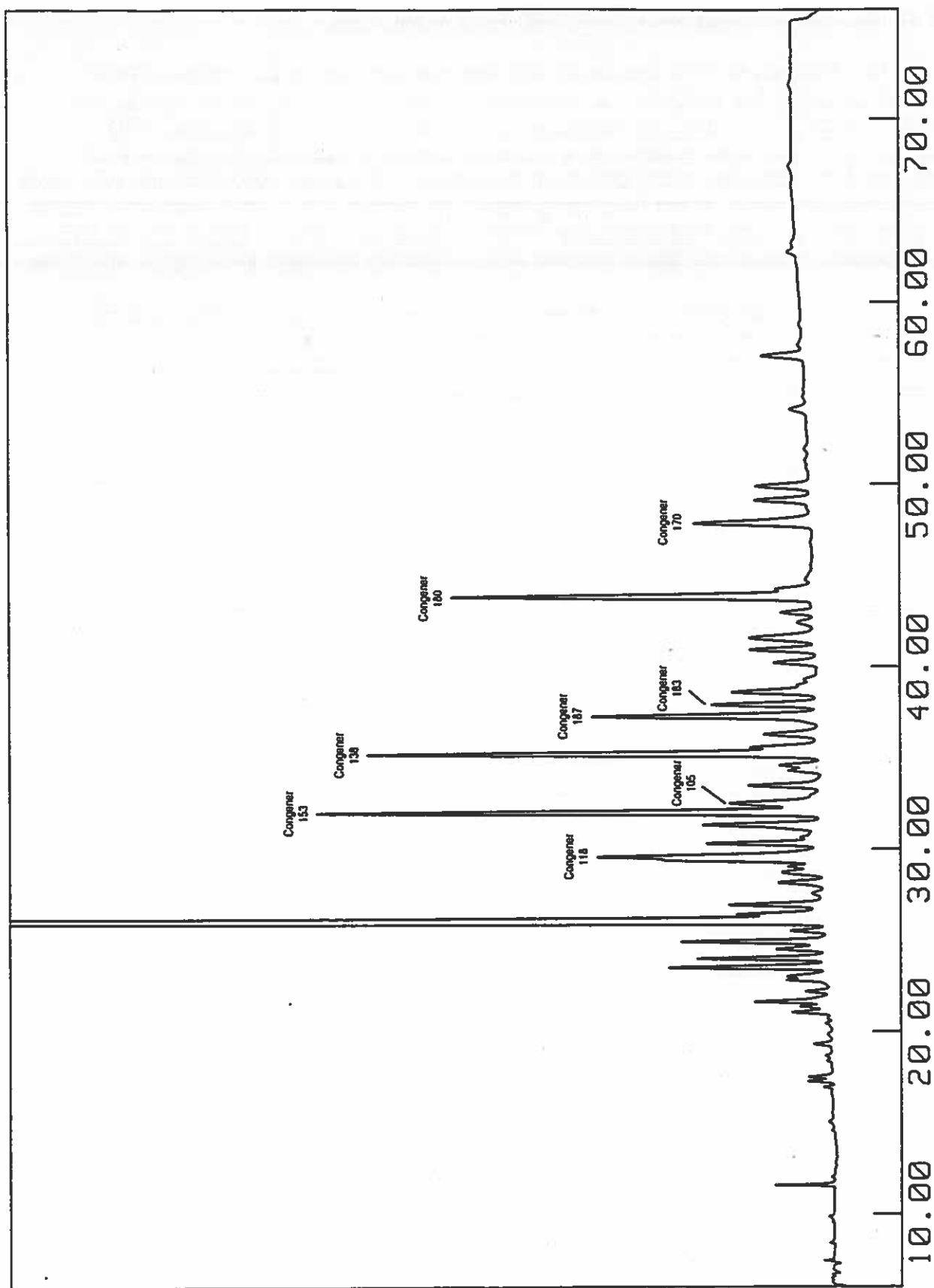


Figure 5. HRGC/ECD chromatogram of sample 91110124.

SECTION 3

RESULTS

This section provides the results of the PCDD, PCDF, and PCB analyses of the eagle egg and quality control samples.

The PCDD, PCDF, and coplanar PCB results from the HRGC/HRMS analysis are discussed in Section 3.1, and the Aroclor results and congener 105 results are discussed in Section 3.2. Internal quality control sample results including method blanks and matrix spikes are presented in Section 3.3. Initial and continuing calibration data are summarized in Section 3.4

3.1 HRGC/HRMS RESULTS—PCDDs, PCDFs, AND COPLANAR PCBs

The results for the 2,3,7,8-substituted PCDD and PCDF isomers and the coplanar PCB congeners from the analysis of the three eagle egg samples are presented in Table 7. The analyte concentrations are given as parts per trillion (ppt or pg/g) based on the lipid content of the samples. For compounds not detected, the calculated detection limit based on the lowest calibration standard is shown in parentheses.

The most toxic PCDD and PCDF isomers, including TCDD, TCDF, PeCDD, and PeCDF, were detected in each of the three eagle samples. Both 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD were detected in each of the three eagle egg samples at significant lipid adjusted concentrations. Non-2,3,7,8-TCDD and PeCDD isomers were not observed. TCDF and PeCDF isomers detected included both 2,3,7,8-substituted isomers and non-2,3,7,8 isomers. Second column confirmation was not included in the fixed price cost to perform this work as indicated in MRI's telefax on August 20, 1992, to Ms. Patricia Dooley from Mr. John Stanley.

During the analysis of the egg samples analytical difficulties were encountered which resulted in low internal quantitation standards for the coplanar PCBs. The amount of native PCBs present was considerable higher than the amount of internal quantitation standard. The concentrations of the native analytes are adjusted for recovery as an isotope dilution calculation, and the data reported are believed to be an accurate assessment of the concentration of coplanar PCBs present in the eagle egg samples.

**TABLE 7. LIPID BASED CONCENTRATIONS OF PCDDs, PCDFs, AND PCBs
IN EAGLE EGG SAMPLES**

	Field ID:	91110122	91110123	91110124
	Extract ID:	11998	11999	12000
	MS File:	L17V15.RPT	L17V16.RPT	L17V17.RPT
	Matrix:	EGG	EGG	EGG
Compound	% Lipid:	4.93	6.25	6.27
PCDD/PCDF		pg/g	pg/g	pg/g
2,3,7,8-TCDF		91.4	246	358
2,3,7,8-TCDD		256	568	543
1,2,3,7,8-PeCDF		ND (197 cdl) ^a	ND (124 cdl)	ND (160 cdl)
2,3,4,7,8-PeCDF		203	333	432
1,2,3,7,8-PeCDD		213	512	549
1,2,3,4,7,8-HxCDF		ND (179 cdl)	ND (120 cdl)	ND (149 cdl)
1,2,3,6,7,8-HxCDF		ND (192 cdl)	ND (125 cdl)	ND (157 cdl)
2,3,4,6,7,8-HxCDF		ND (203 cdl)	201 ^c	243 ^c
1,2,3,7,8,9-HxCDF		ND (210 cdl)	ND (145 cdl)	ND (172 cdl)
1,2,3,4,7,8-HxCDD		ND (189 cdl)	ND (122 cdl)	ND (142 cdl)
1,2,3,6,7,8-HxCDD		ND (194 cdl)	793	658
1,2,3,7,8,9-HxCDD		ND (189 cdl)	ND (122 cdl)	ND (142 cdl)
1,2,3,4,6,7,8-HpCDF		ND (199 cdl)	ND (134 cdl)	ND (170 cdl)
1,2,3,4,7,8,9-HpCDF		ND (201 cdl)	ND (125 cdl)	ND (169 cdl)
1,2,3,4,6,7,8-HpCDD		ND (180 cdl)	159	ND (159 cdl)
1,2,3,4,6,7,8,9-OCDF		ND (381 cdl)	ND (276 cdl)	ND (345 cdl)
1,2,3,4,6,7,8,9-OCDD		1520	1910	ND (345 cdl)
Homologs				
TCDF		1650	434	844
TCDD		256	568	543
PeCDF		1580	881	1310
PeCDD		213	512	549
HxCDF		476	350	483
HxCDD		ND (194 cdl)	824	683
HpCDF		ND (199 cdl)	301	262
HpCDD		ND (180 cdl)	159	ND (159 cdl)
Coplanar PCBs				
3,3',4,4'-Tetra PCB		13,500	19,500	12,900
3,3',4,4',5-Penta PCB		37,300	73,100	57,500
3,3',4,4',5,5'-Hexa PCB		1,510	4,160	3,030
		µg/g	µg/g	µg/g
Aroclor 1260		111 ^b	152	218
2,3,3',4,4'-Penta PCB		3.76	4.36	7.63

^a ND = Not detected. Value in parentheses is calculated detection limit (cdl) based on the lowest calibration standard.

^b Total PCBs calculated relative to Aroclor 1260.

^c A non-2,3,7,8-HxCDF congener coelutes with 2,3,4,6,7,8-HxCDF in samples 9110123 and 91110124.

3.2 HRGC/ECD RESULTS—AROCOR 1260 AND PCB CONGENER 105

The results from the HRGC/ECD analysis of the eagle egg samples are also presented in Table 7. The concentrations ($\mu\text{g/g}$ or ppm) are based on the extractable lipid content of the samples. Total PCB concentrations based on Aroclor 1260 quantitation and congener specific PCB analysis for congener 105 are included.

3.3 QUALITY CONTROL SAMPLE RESULTS

A method blank, unspiked quality control egg sample, and spiked quality control egg samples were included with the analysis of the three eagle egg samples.

3.3.1 PCDD, PCDF, and Coplanar PCB Quality Control Results

Tables 8 and 9 present the quality control sample results for PCDDs, PCDFs, and coplanar PCBs. The method blank was free of PCDDs and PCDFs at the detection limits shown in Table 8, and the percent accuracy results of the egg pool method spiked sample ranged from 118% to 133% recovery.

The accuracy of the native coplanar PCBs spiked into the QC egg matrix are good (98 % to 127%), even though the recoveries of the internal quantitation standards were low. Concentrations of 3,3',4,4'-tetra PCB were detected in the method blank and QC egg sample at levels considerably lower than observed in the eagle samples.

3.3.2 Aroclor 1260 Quality Control Results

The egg sample spiked with Aroclor 1260 showed a method recovery of 108% which indicated acceptable recovery through the sample preparation and analysis procedures. The method blank and unspiked QC egg sample were free of PCB background at an instrumental detection limit $< 0.1 \mu\text{g/g}$ corresponding to 3 g lipid.

3.4 $^{13}\text{C}_{12}$ INTERNAL QUANTITATION STANDARD AND SURROGATE RECOVERIES

The percent recoveries for the carbon-13 internal quantitation standards (IQS) and surrogates are shown in Tables 10 and 11. The recovery objective for this analysis was 25% to 150%. The carbon-13 IQS were added to the samples prior to extraction, and the concentrations of the native compounds were calculated relative to these standards as an isotope dilution technique. The recoveries of the IQS were calculated relative to recovery standards added to the sample extract just before analysis. The Aroclor 1260 results were not corrected for surrogate recoveries.

As discussed earlier, the coplanar IQS recoveries were below the recovery objective. Based on the isotope dilution calculation, the native concentrations may not be adversely affected by these low recoveries. Due to the high concentrations of

TABLE 8. LIPID BASED CONCENTRATIONS (pg/g) of PCDDs AND PCDFs IN
QUALITY CONTROL EGG SAMPLES

Compound	Field ID: Extract ID: MS File: Matrix: % Lipid:	M. Blank 11994 L17V11.RPT EGG N/A	UnSpiked QC Pool 11995 L17V12.RPT EGG 32.2	PCDD/PCDF		Atorol 1260	
				QC Spike 1 11996 L17V13.RPT EGG 33.4	QC Spike 2 11997 L17V14.RPT EGG 33.5	QC Spike 1 spike level	QC Spike 1 % accuracy (c)
2,3,7,8-TCDF		ND (3.23 cdl) ^a	ND (7.28 cdl)	72.4	ND (4.77 cdl)	59.8	121
2,3,7,8-TCDD		ND (2.85 cdl) ^b	ND (7.66 cdl)	73.7	ND (5.23 cdl)	59.8	123
1,2,3,7,8-PeCDF		ND (15.4 cdl)	ND (32.4 cdl)	371	ND (23 cdl)	299	124
2,3,4,7,8-PeCDF		ND (13.1 cdl)	ND (35.2 cdl)	379	ND (23.4 cdl)	299	127
1,2,3,7,8-PeCDD		ND (14.7 cdl)	ND (33.9 cdl)	376	ND (23.9 cdl)	299	126
1,2,3,4,7,8-HxCDF		ND (15.5 cdl)	ND (28.9 cdl)	378	ND (20.2 cdl)	299	127
1,2,3,6,7,8-HxCDF		ND (15 cdl)	ND (30.6 cdl)	380	ND (21 cdl)	299	127
2,3,4,6,7,8-HxCDF		ND (14.7 cdl)	ND (28.4 cdl)	369	ND (23 cdl)	299	123
1,2,3,7,8,9-HxCDF		ND (14.4 cdl)	ND (38.4 cdl)	398	ND (25.5 cdl)	299	133
1,2,3,4,7,8-HxCDD		ND (14.1 cdl)	ND (29.6 cdl)	385	ND (21.6 cdl)	299	129
1,2,3,6,7,8-HxCDD		ND (13.9 cdl)	ND (29 cdl)	387	ND (21.9 cdl)	299	130
1,2,3,7,8,9-HxCDD		ND (14.1 cdl)	ND (29.6 cdl)	342.5	ND (21.6 cdl)	299	115
1,2,3,4,6,7,8-HpCDF		ND (13.5 cdl)	ND (31.8 cdl)	383	ND (22.8 cdl)	299	128
1,2,3,4,7,8,9-HpCDF		ND (13.9 cdl)	ND (31.9 cdl)	389	ND (24.9 cdl)	299	130
1,2,3,4,6,7,8-HpCDD		ND (11.1 cdl)	ND (30.3 cdl)	377	ND (22.8 cdl)	299	126
1,2,3,4,6,7,8,9-OCDF		ND (27.2 cdl)	ND (64.8 cdl)	706	ND (48.7 cdl)	598	118
1,2,3,4,6,7,8,9-OCDD		ND (27.2 cdl)	ND (64.8 cdl)	781	ND (48.7 cdl)	598	131
Homologs							
TCDF		ND (3.23 cdl)	ND (7.28 cdl)	72.4	ND (4.77 cdl)		
TCDD		ND (2.85 cdl)	ND (7.66 cdl)	73.7	ND (5.23 cdl)		
PeCDF		ND (15.4 cdl)	ND (32.4 cdl)	753	ND (23 cdl)		
PeCDD		ND (14.7 cdl)	ND (33.9 cdl)	376	ND (23.9 cdl)		
HxCDF		ND (15.5 cdl)	ND (28.9 cdl)	1380	ND (20.2 cdl)		
HxCDD		ND (13.9 cdl)	ND (29 cdl)	732	ND (21.9 cdl)		
HpCDF		ND (13.5 cdl)	ND (31.8 cdl)	732	ND (22.8 cdl)		
HpCDD		ND (11.1 cdl)	ND (30.3 cdl)	377	ND (22.8 cdl)		

^a NA = Not applicable. Detection limits for method blank are based on 10 g sample size.
^b ND = Not detected. Value in parentheses is calculated detection limit (cdl) based on the lowest calibration standard.
^c % Accuracy = (amount detected in spiked sample/theoretical amount spiked) x 100%.

TABLE 9. LIPID-BASED CONCENTRATIONS OF PCBs IN QUALITY CONTROL EGGS

Compound	Field ID:	M. Blank	QC Pool	QC Spike 1	QC Spike 1 Spike Level pg/g	QC Spike 1 % Accuracy ^c
	Extract ID:	11994	11995	11996		
	MS File:	L17V11.RPT	L17V12.RPT	L17V13.RPT		
Matrix:		EGG	EGG	EGG	pg/g	
		pg/g	pg/g	pg/g		
3,3',4,4'-Tetra PCB		2.36	35.2	153	120	98.3
3,3',4,4',5-Penta PCB		ND (.956) ^a	ND (3.67 mpc) ^b	152	120	127
3,3',4,4',5,5'-Hexa PCB		ND (.721)	ND (3.46)	142	120	119
2,3,3',4,4'-Penta PCB		µg/g	µg/g	QC Spike 2	QC Spike 2	QC Spike 2
Aroclor 1260		<0.03	<0.03	µg/g	Spike Level	% Accuracy
		<0.1	<0.1	.223	µg/g	

^a ND = Compound not detected. Value in parentheses is the detection limit based on 2.5 times the instrumental noise.

^b ND = Compound not detected. The maximum possible concentration (mpc) based on a response that did not meet the qualitative criteria is shown in parentheses.

^c % Accuracy = [(Amount detected in spiked QC sample - amount in unspiked QC sample)/Theoretical amount spiked] x 100%.

TABLE 10. ABSOLUTE RECOVERIES (%) OF ¹³C₁₂ INTERNAL QUANTITATION STANDARDS AND ³⁷Cl CLEANUP STANDARD

Compound	M. Blank												Mean recovery ^a	% RSD ^b	
	Field ID:	11984	QC Pool	QC Spike 1	QC Spike 2	91110122	91110123	91110124							
	Extract ID:	L17V11.RP	11995	11996	11997	L17V15.RPT	L17V16.RPT	L17V17.RPT	12000						
	MS File:	T	L17V12.RPT	L17V13.RPT	L17V14.RPT	L17V15.RPT	L17V16.RPT	L17V17.RPT	EGG	EGG	EGG	EGG			
	Matrix:	EGG	EGG	EGG	EGG	EGG	EGG	EGG	EGG	EGG	EGG	EGG			
% Lipid:	N/A	32.2	33.4	33.5	4.93	6.25	6.27								
¹³ C-2,3,7,8-TCDF	31.0	42.3	48.1	62.5	48.6	59.4	45.2	15.8							
¹³ C-2,3,7,8-TCDD	35.1	40.2	45.1	57.0	45.1	51.5	39.0	14.8							
¹³ C-1,2,3,7,8-PeCDF	32.5	47.5	54.0	64.7	51.4	64.2	49.8	13.4							
¹³ C-2,3,4,7,8-PeCDF	38.3	43.7	54.3	63.8	51.7	63.6	47.7	15.2							
¹³ C-1,2,3,7,8-PeCDD	34.0	45.4	54.4	62.4	51.4	65.4	46.5	15.1							
¹³ C-1,2,3,4,7,8-HxCDF	32.2	53.3	58.9	73.9	56.6	66.4	53.3	13.6							
¹³ C-1,2,3,6,7,8-HxCDF	33.3	50.3	55.0	71.0	52.8	63.6	50.6	14.6							
¹³ C-2,3,4,6,7,8-HxCDF	34.0	54.2	54.8	64.7	50.0	60.3	49.4	10.7							
¹³ C-1,2,3,7,8,9-HxCDF	34.7	40.1	42.9	58.5	48.3	54.8	46.1	14.5							
¹³ C-1,2,3,4,7,8-HxCDD	35.4	52.1	60.9	69.0	53.8	65.3	56.1	11.3							
¹³ C-1,2,3,6,7,8-HxCDD	35.9	53.1	60.4	68.0	52.4	62.2	53.8	10.7							
¹³ C-1,2,3,4,6,7,8-HpCDF	37.1	48.4	52.7	65.5	50.9	59.2	46.6	13.3							
¹³ C-1,2,3,4,7,8,9-HpCDF	36.0	48.3	46.8	59.9	50.4	63.5	47.0	13.7							
¹³ C-1,2,3,4,6,7,8-HpCDD	45.0	50.8	53.1	65.4	56.3	69.0	49.9	13.9							
¹³ C ₁₂ -OCDD	36.7	47.5	50.1	61.2	53.2	57.7	46.1	11.2							
³⁷ Cl-2378TCDD	38.0	40.1	45.5	56.5	46.6	55.0	39.0	15.5							

^a Mean recovery from analysis of three QC egg samples and three eagle egg samples.^b Relative Standard Deviation.

TABLE 11. RECOVERIES (%) OF ¹³C₁₂ PCB INTERNAL QUANTITATION STANDARDS AND SURROGATES

Field ID:	M. Blank	QC Pool	QC Spike 1	QC Spike 2	91110122	91110123	91110124
Extract ID:	11994	11995	11996	11997	11998	11999	12000
MS File:	L17V11.RPT	L17V12.RPT	L17V13.RPT	L17V14.RPT	L17V15.RPT	L17V16.RPT	L17V17.RPT
Compound	EGG	EGG	EGG	EGG	EGG	EGG	EGG
Matrix:	EGG	EGG	EGG	EGG	EGG	EGG	EGG
¹³ C-3,3',4,4'-Tetra PCB	33	20.3	29	28.5	12.4	9.55	7.08
¹³ C-3,3',4,4',5-Penta PCB	25.4	13.1	17.8	19.6	5.81	4.91	2.51
¹³ C-3,3',4,4',5,5'-Hexa PCB	30.4	18.3	25	27.3	12.7	13.1	8.47
Tetrachloro- <i>m</i> -xylene	59.2	62.5	N/A ^a	72.1	68.1	63.1	99.1
Decachlorobiphenyl	73.2	82.3	N/A	84.0	175 ^b	256	411

^a N/A = Not analyzed; QC Spike 1 was designated for PCDD/PCDF analysis.

^b Decachlorobiphenyl was present in the egg samples above the surrogate spike level due to high levels of PCBs in the samples.

coplanar PCB detected in the samples, the sample extracts may have required dilution to a lower concentration if 100% IQS recoveries had been achieved.

The surrogate recoveries for tetrachloro-*m*-xylene and decachlorobiphenyl were acceptable for the method blank and egg QC samples. Decachlorobiphenyl surrogate recoveries for the three eagle egg samples were unacceptably high due to native concentrations of deca-PCB in the samples.

3.5 CALIBRATION DATA

The initial and continuing calibration criteria were met for each day that samples were analyzed. The PCDD and PCDF isomer-specific and total homolog calibration data are summarized in Tables 12 and 13, and the coplanar PCB calibration data are summarized in Table 14.

The Aroclor 1260, surrogate calibration data, and the PCB congener 105 data are included in Table 15.

TABLE 12. PCDD/PCDF ISOMER CALIBRATION CURVE SUMMARY

Compound	Extract ID:		CS1		CS2		CS3		CS4		CS5		Mean	% RSD
	MS File:	L17VQ31.RPT	L17VQ32.RPT	L17VQ41.RPT	L17VQ42.RPT	L17VQ51.RPT	Mean	% RSD	Mean	% RSD	Mean	% RSD		
¹³ C-2,3,7,8-TCDF		1.64	1.58	1.70	1.58	1.77	1.65	4.9	1.65	4.9	1.65	4.9		
¹³ C-2,3,7,8-TCDD		1.12	1.04	1.10	1.07	1.17	1.10	4.5	1.10	4.5	1.10	4.5		
¹³ C-1,2,3,7,8-PeCDF		0.915	0.818	0.916	0.884	1.04	0.915	8.8	0.915	8.8	0.915	8.8		
¹³ C-2,3,4,7,8-PeCDF		1.09	0.979	1.09	1.04	1.26	1.09	9.6	1.09	9.6	1.09	9.6		
¹³ C-1,2,3,7,8-PeCDD		0.504	0.467	0.511	0.478	0.597	0.511	10.0	0.511	10.0	0.511	10.0		
¹³ C-1,2,3,4,7,8-HxCDF		1.11	1.10	1.15	1.13	1.12	1.12	1.7	1.12	1.7	1.12	1.7		
¹³ C-1,2,3,6,7,8-HxCDF		1.16	1.11	1.17	1.11	1.10	1.13	2.9	1.13	2.9	1.13	2.9		
¹³ C-2,3,4,6,7,8-HxCDF		1.13	1.06	1.07	1.04	1.06	1.07	3.2	1.07	3.2	1.07	3.2		
¹³ C-1,2,3,7,8,9-HxCDF		0.847	0.824	0.836	0.837	0.865	0.842	1.8	0.842	1.8	0.842	1.8		
¹³ C-1,2,3,4,7,8-HxCDD		0.815	0.761	0.805	0.826	0.826	0.807	3.3	0.807	3.3	0.807	3.3		
¹³ C-1,2,3,6,7,8-HxCDD		0.977	0.943	0.917	0.893	0.905	0.927	3.6	0.927	3.6	0.927	3.6		
¹³ C-1,2,3,4,6,7,8-HpCDF		0.970	0.952	0.916	0.996	0.970	0.961	3.1	0.961	3.1	0.961	3.1		
¹³ C-1,2,3,4,7,8,9-HpCDF		0.791	0.763	0.759	0.740	0.820	0.775	4.0	0.775	4.0	0.775	4.0		
¹³ C-1,2,3,4,6,7,8-HpCDD		0.905	0.863	0.873	0.905	0.903	0.890	2.3	0.890	2.3	0.890	2.3		
¹³ C ₁₂ -OCDD		0.762	0.688	0.707	0.691	0.776	0.725	5.7	0.725	5.7	0.725	5.7		
³⁷ Cl-2,3,7,8-TCDD		1.12	1.02	1.08	1.05	1.19	1.09	6.1	1.09	6.1	1.09	6.1		
2,3,7,8-TCDF		0.915	0.882	0.857	0.867	0.891	0.882	2.6	0.882	2.6	0.882	2.6		
2,3,7,8-TCDD		1.16	1.04	0.983	1.02	1.05	1.05	6.3	1.05	6.3	1.05	6.3		
1,2,3,7,8-PeCDF		0.913	0.91	0.895	0.908	0.935	0.912	1.6	0.912	1.6	0.912	1.6		
2,3,4,7,8-PeCDF		0.863	0.863	0.879	0.907	0.907	0.884	2.5	0.884	2.5	0.884	2.5		
1,2,3,7,8-PeCDD		1.34	1.35	1.36	1.39	1.45	1.38	3.2	1.38	3.2	1.38	3.2		
1,2,3,4,7,8-HxCDF		1.18	1.16	1.17	1.20	1.21	1.18	1.8	1.18	1.8	1.18	1.8		
1,2,3,6,7,8-HxCDF		1.18	1.19	1.22	1.24	1.28	1.22	3.3	1.22	3.3	1.22	3.3		
2,3,4,6,7,8-HxCDF		1.23	1.18	1.19	1.23	1.25	1.22	2.4	1.22	2.4	1.22	2.4		
1,2,3,7,8,9-HxCDF		1.27	1.22	1.25	1.32	1.30	1.27	3.1	1.27	3.1	1.27	3.1		
1,2,3,4,7,8-HxCDD		0.743	0.854	0.864	0.927	0.952	0.868	9.4	0.868	9.4	0.868	9.4		
1,2,3,6,7,8-HxCDD		0.767	0.937	0.942	0.989	0.977	0.922	9.7	0.922	9.7	0.922	9.7		
1,2,3,7,8,9-HxCDD-1		0.910	1.05	1.03	1.03	1.05	1.01	5.8	1.01	5.8	1.01	5.8		
1,2,3,7,8,9-HxCDD-2		0.759	0.844	0.906	0.953	0.953	0.883	9.3	0.883	9.3	0.883	9.3		
1,2,3,4,6,7,8-HpCDF		1.44	1.47	1.50	1.52	1.52	1.49	2.3	1.49	2.3	1.49	2.3		
1,2,3,4,7,8,9-HpCDF		1.43	1.41	1.48	1.50	1.50	1.46	2.8	1.46	2.8	1.46	2.8		
1,2,3,4,6,7,8-HpCDD		0.904	0.858	0.876	0.909	0.909	0.891	2.6	0.891	2.6	0.891	2.6		
1,2,3,4,6,7,8,9-OCDF		1.09	1.13	1.10	1.17	1.19	1.14	3.8	1.14	3.8	1.14	3.8		
1,2,3,4,6,7,8,9-OCDD		1.36	1.23	1.21	1.23	1.25	1.26	4.8	1.26	4.8	1.26	4.8		

TABLE 13. PCDD/PCDF HOMOLOG CALIBRATION CURVE SUMMARY

Compound	Extract ID:		CS2		CS3		CS4		CS5		Mean	% RSD
	MS File:	L17VQ31.RPT	L17VQ32.RPT	L17VQ32.RPT	L17VQ41.RPT	L17VQ41.RPT	L17VQ42.RPT	L17VQ42.RPT	L17VQ51.RPT	L17VQ51.RPT		
2,3,7,8-TCDF		0.915	0.882	0.882	0.857	0.857	0.867	0.867	0.891	0.891	0.882	2.6
2,3,7,8-TCDD		1.16	1.04	1.04	0.983	0.983	1.02	1.02	1.05	1.05	1.05	6.3
1,2,3,7,8-PeCDF		0.913	0.91	0.91	0.895	0.895	0.908	0.908	0.935	0.935	0.912	1.6
2,3,4,7,8-PeCDF		1.03	1.03	1.03	1.05	1.05	1.07	1.07	1.09	1.09	1.05	2.2
Average PeCDF*											0.983	
1,2,3,7,8-PeCDD		1.34	1.35	1.35	1.36	1.36	1.39	1.39	1.45	1.45	1.38	3.2
1,2,3,4,7,8-HxCDF		1.18	1.16	1.16	1.17	1.17	1.20	1.20	1.21	1.21	1.18	1.6
1,2,3,6,7,8-HxCDF		1.24	1.2	1.2	1.25	1.25	1.21	1.21	1.25	1.25	1.23	1.7
2,3,4,6,7,8-HxCDF		1.25	1.14	1.14	1.11	1.11	1.13	1.13	1.17	1.17	1.16	4.2
1,2,3,7,8,9-HxCDF		0.968	0.915	0.915	0.91	0.91	0.977	0.977	1.00	1.00	0.95	3.7
Average HxCDF											1.13	
1,2,3,4,7,8-HxCDD		0.743	0.689	0.689	0.758	0.758	0.857	0.857	0.868	0.868	0.793	8.7
1,2,3,6,7,8-HxCDD		0.767	0.937	0.937	0.942	0.942	0.989	0.989	0.977	0.977	0.922	8.7
1,2,3,7,8,9-HxCDD		0.829	0.844	0.844	0.906	0.906	0.953	0.953	0.953	0.953	0.914	5.8
Average HxCDD											0.876	
1,2,3,4,6,7,8-HpCDF		1.44	1.47	1.47	1.50	1.50	1.52	1.52	1.52	1.52	1.49	2.1
1,2,3,4,7,8,9-HpCDF		1.17	1.13	1.13	1.22	1.22	1.12	1.12	1.26	1.26	1.18	4.5
Average HpCDF											1.34	
1,2,3,4,6,7,8-HpCDD		0.904	0.858	0.858	0.876	0.876	0.909	0.909	0.909	0.909	0.891	2.6

* The 2,3,7,8 isomer-specific response factors relative to the ¹³C₁₂ IQS specified in EPA Method 8290 were averaged for homolog response factors.

TABLE 14. COPLANAR PCB HRGC/HRMS CALIBRATION SUMMARY

Compound	CS1	CS2	CS3	CS4	CS5	CS6	Mean	% RSD
¹³ C-Tetra-PCB	0.465	0.474	0.531	0.492	0.512	0.519	0.499	5.25
¹³ C-Penta-PCB	1.23	1.24	1.32	1.26	1.19	1.21	1.24	3.65
¹³ c-Hexa-PCB	0.387	0.379	0.438	0.438	0.411	0.419	0.412	6.06
PCB No. 77	2.91	2.75	2.86	2.92	2.85	2.85	2.86	2.12
PCB No. 126	0.664	0.611	0.666	0.662	0.661	0.692	0.659	4.00
PCB No. 169	1.74	1.60	1.94	1.89	1.87	1.90	1.82	7.06

TABLE 15. HRGC/ECD CALIBRATION SUMMARY

Compound	CF Level 1	CF Level 2	CF Level 3	CF Level 4	CF Level 5	CF Mean	% RSD
Aroclor 1260	15165472 ^a	15489552	14954169	13893504	12938046	14488148	7.3
Tetrachloro- <i>m</i> -xylene	76995200	71534400	68419007	76619400		73392017	5.6
Decachlorobiphenyl	40534000	37026000	35270000	40633000		3836570	6.9
2,3,3',4,4'-Penta PCB	8748.7 ^b	8421.9	8960.6			8710.4	3.1

^a Calibration factor for Aroclor 1260 and surrogates based on area/concentration (µg/mL).

^b Calibration factor for 2,3,3',4,4' PCB based on area/concentration (ng/mL).

SECTION 4

DATA PACKET ORGANIZATION

The supporting analytical data for this study are organized into sample preparation, HRGC/HRMS data packets, and HRGC/ECD data packets. The first folder includes sample receipt information, the work plan for sample preparation, and the sample preparation data forms.

The HRGC/HRMS data packet includes copies of MRI'S data reduction procedures for understanding the input and output files used for the computer-generated data reduction. The data reduction files for calibration and samples are provided. The ion plots and area reports for each standard and sample are provided in a separate folder.

The HRGC/ECD data packet includes the analysis sequence and the HRGC/ECD chromatograms with area reports. Data reduction spreadsheets performed using Excel software with a Macintosh SE/30 computer are included. The sample calculations are explained on the spreadsheets.

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